

KERATINOPHILIC AND CYCLOHEXIMIDE RESISTANT FUNGI IN SOILS OF OMAN

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ABSTRACT - Using goat hair fragments as baits at 28°C, 32 keratinophilic cycloheximide resistant species and 1 variety belonging to 14 genera were collected from 50 soil samples gathered from different places of Oman. Numerous keratinophilic fungi were isolated namely *Aphanoascus* sp. teleomorph of *Chrysosporium tropicum*, *A. fulvescens* teleomorph of *C. keratinophilum*, *A. terreus* teleomorph of *C. indicum*, *Arthroderma cuniculi*, *A. lentiginosum* teleomorph of *Trichophyton terrestre*, *A. tuberculata*, *A. curreyi*, *A. ciferri* teleomorph of *C. georgii*, *Chrysosporium carmichaelii*, *C. lucknowense*, *C. pannicola*, *C. prunosum*, *C. asperatum*, *C. xerophilum*, *Trichophyton mentagrophytes*, *T. rubrum*, *Apinisia queenslandica* teleomorph of *C. queenslandicum*, *Myceliophthora vellerea* and *Microsporium gypseum*. Also several other saprophytic and cycloheximide resistant fungi were isolated.

RÉSUMÉ - L'utilisation de fragments de poils de chèvre comme piège à 28°C, a permis d'isoler, à partir de 50 échantillons de sols récoltés à Oman, 32 espèces et une variété, appartenant à 14 genres, de champignons kératinophiles résistants au cycloheximide. Les espèces les plus fréquemment isolées sont *Aphanoascus* sp. téléomorphe de *Chrysosporium tropicum*, *A. fulvescens* téléomorphe de *C. keratinophilum*, *A. terreus* téléomorphe de *C. indicum*, *Arthroderma cuniculi*, *A. lentiginosum* téléomorphe de *Trichophyton terrestre*, *A. tuberculata*, *A. curreyi*, *A. ciferri* téléomorphe de *C. georgii*, *Chrysosporium carmichaelii*, *C. lucknowense*, *C. pannicola*, *C. prunosum*, *C. asperatum*, *C. xerophilum*, *Trichophyton mentagrophytes*, *T. rubrum*, *Apinisia queenslandica* téléomorphe de *C. queenslandicum*, *Myceliophthora vellerea* et *Microsporium gypseum*. De nombreuses autres espèces de champignons saprophytes, résistant au cycloheximide ont également été isolées.

KEY WORDS Keratinophilic fungi, cycloheximide resistant fungi, soil borne fungi

INTRODUCTION

Keratinophilic fungi are of importance and considerable significance and several investigations have been made on the contribution of these in soil of many countries all over the world (Ajello & Ziedberg, 1951, Ajello, 1952, 1954, Ajello et al., 1964, Randhawa & Sandhu, 1965, Ajello & Padhye, 1974, Caretta & Piontelli, 1975, Caretta et al., 1977, Crozier, 1980, Mc-Aleer, 1980, Sur & Gosh, 1980, Calvo et al., 1984, Marsella & Mercantini, 1986, Sundaram, 1987, Chabasse, 1988).

In Arab countries, few surveys were carried out on keratinophilic fungi from soil (Jana et al., 1979, Amer et al., 1975, Abdel Fattah et al., 1982; Abdel Mallek et al., 1989, Youssef et al., 1989, Karam E.-Din et al., 1990; Abdel Hafez et al., 1989a,

1991, El Said, 1993) The present investigation aimed to study intensively composition and frequency of occurrence of keratinophilic fungi in Omani soil

MATERIALS AND METHODS

Fifty soil samples were collected from different localities of Oman, according to the method described by Johnson et al. (1959).

The soil samples were analysed chemically for the estimation of total soluble salts, elements (Ca, Mg, K and Na) and organic matter. A pH-meter (WGPYE model 290) was used for the determination of soil pH. The soil type was determined by the hydrometer method as described by Piper (1955) and most of samples are sandy.

Isolation of Keratinophilic fungi:

The hair baiting technique was employed as recommended by Vanbreuseghem (1952), and as employed by Abdel Fattah et al. (1982). 100g of soil were put in sterile plate and a sufficient quantity of sterile distilled water (about 20-25% moisture content) was added and mixed thoroughly. Pieces of sterile goats hair were sprinkled on the surface of the moistened soil. Two plates were used for each sample, the plates were incubated at 28°C for 6-8 weeks, and the soil in plates were moistened whenever necessary. The moulds which appeared on the baits were transferred to the surface of Sabouraud's dextrose agar medium (Moss & Mc Quown, 1969) which was supplemented with 20 units/ml of sodium penicillin, 40 µg/ml of dihydrostreptomycin and 0.05% cycloneximide (Actidione). Before adding to the agar, the first 2 antibiotics were dissolved separately in sterile distilled water while the third was dissolved in methanol. The plates were incubated at 28°C for 3-4 weeks and the developing colonies were examined and identified.

RESULTS AND DISCUSSION

The soil samples tested were generally poor in organic matter content (0.09-2.16% of dry soil) and their contents in total soluble salts widely ranged between 0.02-19.2%, in Ca 0.02-2.17 mg, Mg 0.02-0.66 mg, K 0.02-0.51 mg, and Na 0.02-2.2 mg/g dry soil. Abdel Fattah (1973) found that the total soluble salts of Egyptian desert soils varies between 0.4-6.6%. The pH values of the soils tested were all in alkaline side (7.3-8.9).

Thirty-two keratinophilic and cycloneximide resistant species in addition to 1 variety which belong to 14 genera were collected from 50 soil samples baited with goat hair fragments at 28°C (Table 1).

Aphanoascus teleomorph of *Chrysosporium* was the most common genus, occurring in 52% of the samples. It was represented by 3 species of which *Aphanoascus* sp. teleomorph of *Chrysosporium tropicum* was the most common species and was represented in 28% of the soil samples. It was dominant species in Italy soils (24.5% of the samples) as recorded by Todaro (1978). It was represented in 20.8 and 12.4% of the soil samples in Marrakesh and Casablanca (Jana et al., 1979). In India, *C. tropicum* occurred in 18% of the soil samples tested (Sur & Ghosh, 1980), in Galapagos Islands in 5.3% (Ajello & Padhye, 1974), in Chilean Andes in 3.9% (Piontelli & Caretta, 1974), in soil of Volcano Etna, 20.5% (Caretta et al., 1977), in Spain in 24% (Cavo et al., 1984), and Yemen in 24% (El-Said, 1993). In Egypt, *C. tropicum* was the most common fungal species recovered by baiting, comprising 36.6% of the total

fungal isolates (Mostafa, 1977) Abdel-Hafez et al (1991) isolated *C. tropicum* in 25.3% of the soil samples collected from Eastern desert in Egypt Abdel-Fattah et al (1982) isolated this species in 11.4% of the soil samples collected from Assiut Governorate

Table 1 Numbers of cases of isolations (NCI out of 50), percentage frequency (%F) and occurrence remark (OK) of fungal genera and species recovered from 50 soil samples baited with goats hair at 28°C.

Genera and species	NCI	%F	OR
<i>Aphanoascus</i>	26	52	H
<i>Aphanoascus</i> sp	14	28	M
<i>A. fulvescens</i> (Cooke) Apinis	10	20	L
<i>A. terreus</i> (Randhawa & Sandhu) Apinis	9	18	L
<i>Arthroderma</i>	19	38	M
<i>A. cuniculi</i> Dawson	7	14	L
<i>A. lentilare</i> Pore, Tsao & Plunkett	5	10	R
<i>A. tuberculata</i> Kuehn	5	10	R
<i>A. curreyi</i> Berk	4	8	R
<i>A. cifferri</i> Varsavsky & Ajello	2	4	R
<i>Chrysosporium</i>	18	36	M
<i>C. carmichaelii</i> Van Oorschot	6	12	L
<i>C. lucknowense</i> Ga.g	5	10	R
<i>C. pannicola</i> (Corda) Van Oorschot & Stalpers	5	10	R
<i>C. pruinosum</i> Gilman & Abbott	5	10	R
<i>C. asperatum</i> J.W. Carmichael	3	6	R
<i>C. xerophilum</i> Pitt	3	6	R
<i>Aspergillus</i>	17	34	M
<i>A. flavus</i> Link	8	16	L
<i>A. fumigatus</i> Fresenius	6	12	L
<i>A. ustus</i> (Banier) Thom & Church	6	12	L
<i>A. flavus</i> var <i>columnaris</i> Raper & Fennell	5	10	R
<i>A. terreus</i> Thom	4	8	R
<i>A. niger</i> Van Tieghem	3	6	R
<i>Trichophyton</i>	11	22	L
<i>T. mentagrophytes</i> (Robin) Blanchard	6	12	L
<i>T. rubrum</i> (Castellani) Sabouraud	5	10	R
<i>Penicillium</i>	7	14	L
<i>P. chrysogenum</i> Thom	6	12	L
<i>P. funiculosum</i> Thom	1	2	R
<i>P. puberulum</i> Banier	1	2	R
<i>Apinisia queenslandica</i> Apinis & Rees	3	6	R
<i>Cunninghamella echinulata</i> Thaxter	3	6	R
<i>Fusarium</i>	3	6	R
<i>F. oxysporum</i> Shelecht	2	4	R
<i>F. moniliforme</i> Sheldon	1	2	R
<i>Macrophomina phaseolina</i> (Tassi) Goid	3	6	R
<i>Mucor racemosus</i> Fresenius	2	4	R
<i>Verticillium laterium</i> Berkeley	2	4	R
<i>Mycelophthora vellerea</i> (Sacc. & Speg.) Van Oorschot	2	4	R
<i>M. rosporum gypseum</i> (Bodin) Guat & Grigorakis	1	2	R

Occurrence remark H - high occurrence between 25 to 50 cases (out of 50 samples) M - moderate occurrence, between 13 to 24 cases L - low occurrence, between 6 to 12 cases R - rare occurrence, between 1 to 5 cases

A. fulvescens teleomorph of *C. keratinophilum* was the second most frequent fungal species and was encountered in 20% of the soil samples tested. *C. keratinophilum* emerged from 6% on children playgrounds sand samples (Bojanovsky et al., 1979), from 13.2% of soil samples in W Germany (Meissner & Qadripur, 1983), from 16.9% of soils of the Volcano Etna (Caretta et al., 1977), from 10% of the screened soils of Spain (Calvo et al., 1984). In Yemen this species represented 14% of the soil samples as recorded by El-Said (1993). In Egypt, Abdel Fattah et al. (1982) found this species emerged in 27.1% of soil samples collected from Assiut Governorate, but Abdel Hafez et al. (1991) recovered this species from Egyptian soils baited with animal hair (20% of the soil samples tested).

A. terreus teleomorph of *C. indicum* was the third most frequent fungal species and was represented in 18% of the soil samples, whereas it was the most frequent species in soil samples collected from Yemen (El-Said, 1993) and in cultivated soils collected from Upper Egypt (Abdel Fattah et al., 1982), but it was less frequent in Egyptian soils tested by Mostafa (1977), Abdel-Fattah et al. (1982) and Abdel-Hafez et al. (1991). In India, it emerged from 31.3% of the soil samples (Sur & Ghosh, 1980), in mountains localities in the Chilean Andes, from 19.8% (Piontelli & Caretta, 1974), in Galapagos Islands from 2.6% (Ajello & Padhye, 1974), and Spain, from 4% (Calvo et al., 1984) of the soil samples tested.

Arthroderma was the second most frequent genus and was encountered in 38% of the samples tested. From the genus 5 species were collected of which *A. concinnum* was the most common species. The remaining *Arthroderma* species were scarcely recovered and these were *A. lenticularum* teleomorph of *Trichophyton terrestre*, *A. tuberculata* and *A. ciferri* teleomorph of *C. georgii*. The above species were also isolated from soil samples collected from Yemen (El-Said, 1993) and Egypt (Abdel Hafez et al., 1989a, 1991).

Chrysosporium occupied the third place with regard to the number of cases of isolation of fungal genera and it recovered from 36% of the samples examined. Six species of *chrysosporium* were isolated and these were *C. carmichaelii* (12%), *C. lucknowense* (10%), *C. pannicola* (10%), *C. prunosum* (10%), *C. asperatum* (6%) and *C. xerophilum* (6%). All the above species were isolated from the soil samples of Yemen by El-Said (1993) and were emerged from 10, 10, 12, 12, 4 and 10%, respectively. In Egypt, *C. asperatum* and *C. pannicola* were isolated from Egyptian soils by Abdel-Hafez et al. (1989a, 1991).

Aspergillus (5 species + 1 variety) occupied the fourth place according to the number of cases of isolation of fungal on genera and it encountered in 34% of the soil samples. Among *Aspergillus* species, the most commonly collected were *A. flavus*, *A. fumigatus* and *A. ustus*. The remaining *Aspergillus* species were isolated with rare frequency of occurrence and these were *A. flavus* var. *columnaris*, *A. terreus* and *A. niger*. Aspergillosis due to *A. fumigatus* and *A. flavus* has a world-wide distribution (Frey et al., 1979). Most of the above species had been previously encountered, but with different incidences from various types of soil from many parts of the world (Sundaram, 1987; Abdel-Hafez et al., 1989a, El-Said, 1993).

Trichophyton encountered from 22% of the samples tested. From the genus 2 species were collected of which *T. mentagrophytes* was common and recovered from 12% of the samples. It is a human and animal dermatophyte (Nawok, 1970, Fleming, 1975, Frey et al., 1979). It emerged from 1% of sand samples from children's playgrounds in Germany (Bojanovsky et al., 1979), from 68% of soil samples in Kuwait (Amer et al., 1975) from 3% in England (Baxter, 1969) from 12% in Yemen (El-Said, 1993) and from 6% in Egypt (Abdel-Hafez et al., 1989a). *T. rubrum* was less frequent

Penicillium emerged in 14% of the soil examined. It was represented by 3 species: *P. chrysogenum*, *P. funiculosum* and *P. puberulum*. Abdel-Hafez et al. (1989b) isolated *P. chrysogenum* from the mud of Ibrahimia (Egypt). El-Said (1993) isolated all the above species from the soil samples of Yemen.

The remaining isolated 8 genera and 9 species were recovered in rare frequencies as present in Table 1.

Present results reveal that there is no correlation between the distribution and occurrence of keratinophilic and cycloheximide resistant fungi and soil textures or site of samples. But soil samples with low levels of total soluble salts coincided with a wide range of genera and species and vice versa, this due to most of these fungi are highly sensitive to high salinity. Abdel-Hafez et al. (1989a) found that soil samples collected from salt marshes in Sinai Peninsula are free from keratinophilic fungi. *Aphanoascus* teleomorph of *Chrysosporium* was the most frequent keratinophilic genus in the soil samples from Oman as in case of other Egyptian soil collected from Delta area, Upper Egypt, Sinai and Eastern Desert (Abdel-Fattah et al., 1982, Mostafa, 1977; Abdel-Hafez et al., 1989a, 1991). This results agree with those which were recorded by El-Said (1993) in the soil samples of Yemen.

Comparison between the present results and lists of keratinophilic and cycloheximide resistant fungi recovered from soils collected from Egypt (Abdel-Fattah et al., 1982; Mostafa, 1977, Abdel Hafez et al., 1989, 1991) and Yemen (El-Said, 1993) reveal that there is no keratinophilic or cycloheximide resistant fungi characteristic of Omani soils. But, these lists may differ in the order of frequency of occurrence of some fungi.

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